

AP20 Rec'd PCT/PTO 13 JUL 2006

NEB-236-PCT.ST25.txt  
SEQUENCE LISTING

<110> New England Biolabs, Inc.  
Guan, Chudi  
Kumar, Sanjay  
Kucera, Rebecca

<120> Modulation of Enzyme Activity by Changing Reciprocal  
Stereo-Geometric Positions of Two Catalytic Centers in an Enzyme

<130> NEB-236-PCT

<150> 60/524,123

<151> 2003-11-21

<160> 25

<170> PatentIn version 3.2

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<212> DNA

<213> unknown

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gtgccttatg taattccggc gagcaatcac acttacactc cagacttctt acttccaaac	180
ggatatattcg ttgagacaaa gggctctgtg gaaagcgatg atagaaagaa gcacttatta	240
attagggagc agcaccgccga gctagacatc cgtattgtct tctcaagctc acgtactaag	300
ttatacaaag gttctccaac gtcttatgga gagttctgcg aaaagcatgg tattaagttc	360
gctgataaac tgatacctgc tgagtggata aaggaacca agaaggaggt cccctttgat	420
agattaaaaa ggaaaggagg aaagaaataa	450

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## NEB-236-PCT.ST25.txt

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&lt;400&gt; 8

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aaagtcgctt atgtaattag caatcacact tacact 36

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<213> unknown

<220>  
<223> enterobacteria phage T7

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Met Ala Gly Tyr Gly Ala Lys Gly Ile Arg Lys Val Gly Ala Phe Arg  
1 5 10 15

## NEB-236-PCT.ST25.txt

Ser Gly Leu Glu Asp Lys Val Ser Lys Gln Leu Glu Ser Lys Gly Ile  
20 25 30

Lys Phe Glu Tyr Glu Glu Trp Lys Val Pro Tyr Val Ile Pro Ala Ser  
35 40 45

Asn His Thr Tyr Thr Pro Asp Phe Leu Leu Pro Asn Gly Ile Phe Val  
50 55 60

Glu Thr Lys Gly Leu Trp Glu Ser Asp Asp Arg Lys Lys His Leu Leu  
65 70 75 80

Ile Arg Glu Gln His Pro Glu Leu Asp Ile Arg Ile Val Phe Ser Ser  
85 90 95

Ser Arg Thr Lys Leu Tyr Lys Gly Ser Pro Thr Ser Tyr Gly Glu Phe  
100 105 110

Cys Glu Lys His Gly Ile Lys Phe Ala Asp Lys Leu Ile Pro Ala Glu  
115 120 125

Trp Ile Lys Glu Pro Lys Lys Glu Val Pro Phe Asp Arg Leu Lys Arg  
130 135 140

Lys Gly Gly Lys Lys  
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<210> 13  
<211> 151  
<212> PRT  
<213> unknown

<220>  
<223> yersinia pestis phage phiA1122

<400> 13

Met Ala Gly Thr Tyr Ala Ala Arg Gly Ile Arg Lys Val Gly Thr Phe  
1 5 10 15

Arg Ser Gly Leu Glu Asp Lys Val Ser Lys Gln Leu Glu Gly Lys Gly  
20 25 30

Ile Lys Phe Asp Tyr Glu Leu Trp Lys Ile Pro Tyr Val Val Pro Ala  
35 40 45

Ser Asn His Val Tyr Thr Pro Asp Phe Leu Leu Pro Asn Gly Ile Phe  
50 55 60

## NEB-236-PCT.ST25.txt

Ile Glu Thr Lys Gly Leu Trp Glu Ser Asp Asp Arg Lys Lys His Leu  
65 70 75 80

Leu Ile Arg Glu Gln Phe Pro Glu Leu Asp Ile Arg Leu Val Phe Ser  
85 90 95

Ser Ser Arg Thr Lys Leu Tyr Lys Gly Ser Pro Thr Ser Tyr Gly Glu  
100 105 110

Trp Cys Glu Lys His Gly Ile Leu Phe Ala Asp Lys Leu Ile Pro Val  
115 120 125

Glu Trp Leu Lys Glu Pro Lys Lys Glu Val Pro Phe Asp Arg Leu Lys  
130 135 140

Gln Ala Lys Gly Gly Lys Lys  
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<212> PRT  
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<220>  
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Arg Ser Gly Leu Glu Asp Lys Val Ser Lys Gln Leu Glu Ser Lys Gly  
20 25 30

Ile Lys Phe Asp Tyr Glu Leu Trp Arg Ile Pro Tyr Val Ile Pro Ala  
35 40 45

Ser Asp His Leu Tyr Thr Pro Asp Phe Leu Leu Pro Asn Gly Ile Phe  
50 55 60

Ile Glu Thr Lys Gly Leu Trp Asp Ser Asp Asp Arg Lys Lys His Leu  
65 70 75 80

Leu Ile Arg Glu Gln His Pro Glu Leu Asp Ile Arg Leu Val Phe Ser  
85 90 95

Ser Ser Arg Ser Lys Leu Tyr Lys Gly Ser Pro Thr Ser Tyr Ala Glu  
100 105 110

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Trp Cys Glu Lys His Gly Ile Leu Phe Ala Asp Lys Leu Ile Pro Val  
           115                                  120                                  125

Glu Trp Leu Lys Glu Pro Lys Lys Glu Val Pro Phe Asp Lys Phe Lys  
           130                                  135                                  140

Thr Lys Lys Gly Val Lys Lys Asn Gly  
           145                                  150

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Ser Gly Leu Glu Asp Lys Val Ser Lys Gln Leu Glu Ser Lys Gly Ile  
                                   20                                  25                                  30

Lys Phe Asp Tyr Glu Leu Trp Arg Ile Pro Tyr Val Ile Pro Glu Ser  
                                   35                                  40                                  45

Asp His Leu Tyr Thr Pro Asp Phe Leu Leu Pro Asn Gly Ile Phe Ile  
           50                                  55                                  60

Glu Thr Lys Gly Leu Trp Asp Ser Asp Asp Arg Lys Lys His Leu Leu  
   65                                  70                                  75                                  80

Ile Arg Glu Gln His Pro Glu Leu Asp Ile Arg Leu Val Phe Ser Ser  
                                   85                                  90                                  95

Ser Arg Ser Lys Leu Tyr Lys Gly Ser Pro Thr Ser Tyr Gly Glu Trp  
                                   100                                  105                                  110

Cys Glu Lys His Gly Ile Leu Phe Ala Asp Lys Leu Ile Pro Val Ala  
           115                                  120                                  125

Gly Val Lys Glu Pro Lys Lys Glu Val Pro Phe Asp Lys Phe Lys Thr  
           130                                  135                                  140

Lys Lys Gly Val Lys Lys Asn Gly  
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 <213> unknown

## NEB-236-PCT.ST25.txt

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&lt;223&gt; pseudomonas phage gh-1

&lt;400&gt; 16

Met Ala Tyr Ala Gly Pro Lys Gly Ala Arg Thr Gly Ala Phe Arg Ser  
 1 5 10 15

Gly Leu Glu Asp Arg Asn Ala Lys His Met Asp Lys Leu Gly Val Lys  
 20 25 30

Tyr Asp Phe Glu Arg Phe His Ile Asn Tyr Val Val Pro Ala Arg Asp  
 35 40 45

Ala Lys Tyr Thr Pro Asp Phe Val Leu Ala Asn Gly Ile Ile Ile Glu  
 50 55 60

Thr Lys Gly Ile Trp Glu Val Asp Asp Arg Lys Lys His Leu Leu Ile  
 65 70 75 80

Arg Glu Gln Tyr Pro Asp Leu Asp Ile Arg Leu Val Phe Ser Asn Ser  
 85 90 95

Asn Ser Lys Ile Tyr Lys Gly Ser Pro Thr Ser Tyr Ala Asp Phe Cys  
 100 105 110

Thr Lys His Gly Ile Gln Phe Ala Asp Lys Leu Val Pro Arg Asp Trp  
 115 120 125

Leu Lys Glu Ala Arg Lys Glu Ile Pro Gln Gly Val Leu Val Pro Lys  
 130 135 140

Lys Gly Gly  
 145

&lt;210&gt; 17

&lt;211&gt; 141

&lt;212&gt; PRT

&lt;213&gt; unknown

&lt;220&gt;

&lt;223&gt; pseudomonas putida KT2440

&lt;400&gt; 17

Met Gly Leu Lys Tyr Gly Phe Arg Ser Gly Leu Glu Glu Arg Ala Ala  
 1 5 10 15

Asp Gln Leu Thr Ala Val Gly Met Gly Phe Thr Phe Glu Ser Leu Val  
 20 25 30

## NEB-236-PCT.ST25.txt

Val Pro Tyr Thr Arg Pro Ala Lys Val His Lys Tyr Thr Pro Asp Phe  
35 40 45

Ala Leu Ala Asn Gly Ile Ile Val Glu Thr Lys Gly Arg Phe Leu Thr  
50 55 60

Glu Asp Arg Gln Lys Gln Leu Leu Val Lys Ala Gln His Pro Glu Leu  
65 70 75 80

Asp Val Arg Phe Val Phe Ser Asn Ser Lys Thr Lys Ile Asn Lys Arg  
85 90 95

Ser Thr Thr Thr Tyr Ala Asp Trp Cys Ser Lys Asn Gly Phe Gln Tyr  
100 105 110

Ala Asp Lys Leu Val Pro His Ala Trp Leu Asn Glu Pro Val Asn Glu  
115 120 125

Ala Ser Leu Ser Ile Ile Lys Gly Leu Ser Lys Glu Lys  
130 135 140

<210> 18  
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<212> PRT  
<213> unknown

<220>  
<223> roseophage SI01

<400> 18

Met Leu Asn Ser Lys Ser Ser Thr Arg Lys Arg Ala Leu Lys Ala Gly  
1 5 10 15

Tyr Arg Ser Gly Leu Glu Glu Gln Thr Ala Lys Asp Leu Lys Lys Arg  
20 25 30

Lys Val Leu Phe Thr Tyr Glu Glu Thr Lys Ile Lys Trp Leu Asp Ser  
35 40 45

Lys Val Arg Thr Tyr Thr Pro Asp Phe Val Leu Pro Asn Gly Val Ile  
50 55 60

Ile Glu Thr Lys Gly Arg Phe Val Ala Ala Asp Arg Arg Lys His Leu  
65 70 75 80

Glu Ile Gln Lys Gln Phe Gly Thr Leu Tyr Asp Ile Arg Phe Val Phe  
85 90 95



NEB-236-PCT.ST25.txt

Thr Asn Ser Lys Ala Lys Leu Tyr Lys Gly Ala Lys Ser Ser Tyr Ala  
                   100                  105                  110

Asp Trp Cys Asn Lys His Gly Phe Leu Tyr Ala Asp Lys Thr Ile Pro  
           115                  120                  125

Glu Asp Trp Leu Asn Glu  
       130

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 <223> mismatch

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## NEB-236-PCT.ST25.txt

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<220>  
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 <223> mismatch

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<210> 24  
 <211> 149  
 <212> PRT  
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<220>  
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 1 5 10 15

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Met Val Gly Tyr Gly Val Lys Gly Ile Arg Lys Val Gly Ala Phe Arg
1          5          10          15
Ser Gly Leu Glu Asp Lys Val Ser Lys Gln Leu Glu Ser Lys Gly Ile
20          25          30
Lys Phe Glu Tyr Glu Glu Trp Lys Val Pro Tyr Val Ile Pro Ala Ser
35          40          45
Asn His Thr Tyr Thr Pro Asp Phe Leu Leu Pro Asn Gly Ile Phe Val
50          55          60
Glu Thr Lys Gly Leu Trp Glu Ser Asp Asp Arg Lys Lys His Leu Leu
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NEB-236-PCT.ST25.txt

65 70 75 80

Ile Arg Glu Gln His Pro Glu Leu Asp Ile Arg Ile Val Phe Ser Ser  
85 90 95

Ser Arg Thr Lys Leu Tyr Lys Gly Ser Pro Thr Ser Tyr Gly Glu Phe  
100 105 110

Cys Glu Lys His Gly Ile Lys Phe Ala Asp Lys Leu Ile Pro Ala Glu  
115 120 125

Trp Ile Lys Glu Pro Lys Lys Glu Val Ser Phe Asp Arg Leu Lys Arg  
130 135 140

Lys Gly Gly Lys Lys  
145